A Blueprint for Drug/Diagnostic Co-Development: Next-Generation Sequencing (NGS) in Oncology

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INTRODUCTION

There has been rapid improvement and implementation of Next Generation Sequencing (NGS) platforms over the last several years. Despite limited progress with reimbursement for NGS-based cancer panels, they are increasingly being utilized to determine treatment options for oncology patients, thereby suggesting the clinical importance of this new approach. Because of this, it is critical that an appropriate regulatory pathway be developed to ensure these panels are reviewed by the US Food and Drug Administration (FDA) and can be amended via an expeditious review process to enable incorporation of the new marker into clinical practice.

Use of a broad cancer panel is currently being demonstrated in the Lung-MAP[1] clinical trial, where numerous companies have come together to create a multi-drug, multi-arm, biomarker-driven clinical trial for patients with advanced squamous cell lung cancer. Genomic profiling via a comprehensive NGS panel (Foundation One™) and a MET IHC assay is used to match patients to one of several different investigational treatments that are designed to target the genomic alterations found to be driving the growth of their cancer. FDA has supported this trial design with creative and rapid approaches to review both the drugs and diagnostic platforms used in the trial. The Lung-MAP trial is expected to provide data that could support FDA approval of both the drug and associated companion diagnostic. In addition to the Lung-MAP trial, other examples of innovative trial designs include initiatives by the CRUK, the WIN Consortium, and NCI-MATCH. These types of trials serve as a paradigm for the approval of an NGS platform and/or assay as a companion diagnostic for multiple therapeutics.

If this new paradigm proves successful, multiple challenges inherent in Companion Diagnostic (CoDx) development today may be overcome, as exemplified in the situations below:

Example 1: A pharmaceutical company utilizes a large panel of genomic markers during early stage trials in a molecular subtype of cancer for which there is no approved therapy. These markers are not used for patient selection at this stage. Data is collected for research purposes to evaluate if a marker may be relevant for identifying patients with improved response to the drug. During the analysis, patients with a specific biomarker display a dramatically better response to the drug than patients without the marker. The panel assay that was used during this trial is a laboratory developed test (LDT) and the lab that created the panel test is either not interested or it may not be cost-effective to proceed with a CoDx product. The drug company embarks on a path to develop a simpler PCR based assay to test for the marker. After reviewing multiple proposals from partners, it will take at least 18 months to have an assay ready for the pivotal trial; approximately a 9-month delay compared to the timeline for the drug to be ready. If FDA-cleared actionable NGS panels were available, it is possible this type of delay and the need for a switch to a different assay could be avoided.

Example 2: Several drug companies are moving forward with products targeted to a specific marker. Each drug company partners with a different diagnostic company to develop an assay. All of the drugs make it through clinical trials, and three separate companion diagnostics with similar, but not congruent, performance characteristics are approved by FDA. Even though each diagnostic product is labeled for use with a specific drug, it is likely that having multiple assays in addition to the already available LDTs will create a confusing situation for both doctors and laboratories. A more standard set of actionable cancer NGS panels may be a way to avoid this situation.

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[1] http://www.lung-map.org/

Example 3: A pharmaceutical company is required to move forward with an in vitro diagnostic (IVD) CoDx to register their product. Upon successful registration and IVD product launch, multiple LDTs appear on the market combining these same markers into multi-analyte panels. Hospitals and academic centers will use the most cost effective test and have incentives to offer such capabilities internally. Even though a specific drug label might state that the drug must be used with an FDA-approved test, the increased use of NGS panels across centers in the US may lead to use of targeted agents in patients identified with these non-standard tests that may or may not identify the same population for whom the drug product was approved and may not have the same (predictive) clinical utility as the FDA-approved test.

Example 4: In the context of increasing adoption of NGS in the community, a patient's tumor may be profiled at any time in the disease course, such as at diagnosis. Future medical need and treatment decisions for that individual at relapse may be called for with existing or novel targeted therapies guided by the presence of a specific biomarker but no current tissue is accessible. Having the past genetic profile based on a FDA-cleared actionable panel would provide higher confidence information for the treating physician.

Challenges

Currently, all of the marketed oncology panels have been developed by laboratories and validated pursuant to the CLIA regulations as LDTs – i.e., no FDA clearance or approval, though moving forward, FDA oversight may be required in a risk-based fashion[2]. Given the rapid pace of drug development that relies on accurate identification of molecular markers to determine whether a drug is appropriate for a particular patient, the current one drug/one test paradigm is inefficient and unlikely to be acceptable in the clinic for much longer. The current challenges in this area include:

• Independent development of each oncology panel by separate organizations with little or no standardization of markers or performance characteristics between the tests leading to heterogeneous patient selection within defined marker sets

• Different orthogonal reference methods for validation exist for each test making it difficult to draw comparisons even when looking at two sets of validation data side-by-side

• Unclear process for determining the appropriate reference standards. FDA tends to prefer bi-directional Sanger sequencing as a gold standard (although exceptions exist) but given that new technologies may be more sensitive than bi-directional Sanger sequencing an updated process for selecting reference standards needs to be agreed upon

• Unpublished validation data

• Lack of proficiency test guidance for NGS panels

• Heterogeneity in the quantity and quality of the biopsy material available to support the one-test/one-drug model

• Accurate determination of the fraction of malignant cells in input DNA over the total of malignant and non-malignant nucleated cells. Currently, this is achieved by a pathology estimate and may be inadequately accounted for in the context of somatic mutation analysis and validation by NGS

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[2] http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf

- Different underlying platform technologies that have individual strengths and weakness

- "Equal" regulatory consideration given to all oncology markers, in that all are assumed to need a PMA when used in the context of determining treatment (applies to predictive and prognostic markers)

- Reimbursement challenges will remain until standardization of panel content and agreed upon approaches for demonstrating performance are established (i.e. payment for select biomarkers on a panel based on levels of clinical utility)

- Need for the ability to update panels with newly relevant content

A community-wide approach to address these challenges is what brings us together to discuss how to best move towards agreement on regulatory pathways, promoting and implementing standardization of panel content and performance protocols, and reimbursement decisions to ensure patients are receiving accurate, consistent results no matter which oncology panel is utilized. The NGS Working Group, comprised of representatives from the academic, drug development, and government sectors, would like to focus on five proposals that take steps to address these challenges and facilitate development and use of cancer panels. These proposals are related to the following goals to identify existing challenges and opportunities to optimize processes associated with:

Forum Goals

- Regulatory considerations and strategies for FDA approval of an NGS platform to be used as a companion diagnostic in oncology

- Optimal performance characteristics and standardization of markers between different NGS tools and transparent reimbursement strategies

- The potential for a comprehensive NGS tool capable of querying all known "actionable" markers and prognostic genetic markers to serve as a CoDx for all cancer patients in order to streamline development, investigation, regulation and clinical application

Glossary of Terms

The following key terms were defined by the participants of this working group, in the context of this document:

Actionable Marker: Documented alterations with supporting data that allow for a benefit-risk assessment of treatment choice, linking the patient to a FDA approved drug, on- or off-label, a drug in the investigational phase of development, or an established prognostic outcome. Some markers may be specific (e.g., BRAF V600E), while others may represent a functional group (e.g., alterations in Exon 19 of the EGFR gene). For the purposes of this paper, the focus was on targeted sequencing only (i.e., no consideration of whole genome or whole exome sequencing; thus, incidental findings were not considered in this paper).

Cancer Panel: Panels are a comprehensive (i.e., point mutations, deletions, amplifications, fusions) selection of multi-marker gene assays of cancer driver alterations as opposed to traditional single-marker assays.

Standardization: This working group seeks to define 'what to measure' and not the degree to or performance of the measurements (i.e., level of sensitivity). In other words, standardization in the context of this paper is only agreement on the nature of markers and the performance characteristics of assays to be evaluated on those markers, not a prescribed performance level an assay must attain. The working group has provided suggestions about how to determine appropriate reference standards so that performance may be appropriately assessed.

To date, two panels have been developed for actionable genetic panels in other therapeutic areas. The existing panels may serve as useful models in considering the development of an actionable cancer panel:

1) **Cystic Fibrosis (CF) panels**
   This approach would be similar to the development of molecular assays currently used in the diagnosis and management of cystic fibrosis (CF products utilize a common set of markers as defined by the American College of Medical Genetics and Genomics and American College of Obstetricians and Gynecologists). Although CF testing may in some cases drive therapeutic choices, the information from genetic testing, stored in the patient's medical record, is used more broadly by physicians when treating a patient. Given the existence of FDA cleared CF panels, drug approval targeted to a specific CF genetic marker (Vertex's Kalydeco) did not require the approval of an additional companion diagnostic. Further, this exemption is relevant in that analytical studies for 510(k) cleared CF genetic panels have included the use of contrived specimens to evaluate performance for rare mutations. Since a similar situation is emerging in oncology (i.e., standard of care including testing for a variety of genetic markers and the need for creative approach to analytical testing) it seems appropriate to consider whether this model could be adapted for oncology, particularly when considering rare tumor types or subpopulations.

2) **Microbiology panels**
   Another existing paradigm to consider when discussing regulatory pathways for a molecular oncology panel is microbiology pathogen panels. Non-molecular panels of products for identification of various organisms are currently regulated as Class I exempt products. If information regarding drug resistance of any of these organisms is offered, the classification of the product moves to Class II and requires a 510(k). In addition, microbiology panels based on molecular technology have been cleared via the 510(k) pathway. This model can be extended even to consider which organisms in microbiology need to have more rigorous regulatory pathways. Pathogens that can cause significant disease are regulated as Class III, PMA devices. Class III microbiology targets include HIV, HBV and HCV. It may be possible to extend this existing risk-based approach to oncology targets in that markers for efficacy of drugs may be appropriate to regulate differently than those associated with safety of particular compounds. In addition, more novel approaches such as algorithms designed to choose a particular drug may also be regulated at a higher level on a case-by-case basis. It's interesting to note that at one point microbiology identification panels were regulated as Class III medical devices but were down classified as the technology and use of these panels became standard.

In addition to these two models, the working group would like to draw attention to the intended use of the Qiagen *therascreen* EGFR assay[3]. This approval highlights a creative approach for an assay that has shown clinical utility for multiple mutation classes, and has content that has been analytically demonstrated, but importantly has not been demonstrated to have clinical utility for every mutation. This will be an important precedent to consider in the context of the proposals in this paper.

[3] http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm

The therascreen EGFR RGQ PCR Kit is a real-time PCR test for the qualitative detection of exon 19 deletions and exon 21 (L858R) substitution mutations of the epidermal growth factor receptor (EGFR) gene in DNA derived from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tumor tissue. The test is intended to be used to select patients with NSCLC for whom GILOTRIF (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated. Safety and efficacy of GILOTRIF (afatinib) have not been established in patients whose tumors have L861Q, G719X, S7681, exon 20 insertions, and T790M mutations, which are also detected by the therascreen EGFR RGQ PCR Kit.

Proposals

1. **Proposal:** Define a regulatory pathway for cancer panels intended to identify actionable oncogenic alterations that allows flexibility in the appropriate FDA medical device pathway. Such a pathway could be based on risk classification of different panel components and use the PMA and the 510(k) route depending on the specific marker.

Molecular markers are utilized in a variety of ways, not just for directing specific treatments. As noted above, cancer panels are very quickly gaining adoption in the care for oncology patients, especially within the largest academic cancer centers. As a result, it is critical that an appropriate regulatory pathway be made available to ensure FDA reviews these panels. The NGS Working Group encourages discussion on whether pathways other than Class III PMA are possible (i.e., Class II, 510(k) pathway), such that drug approval based on a class II marker could use the 510(k) route, while that based on a class III marker could use the PMA route. Risk determination for individual markers on a panel could be established based on the classification system defined in proposal 3 with 'Known Actionable' markers being classified as class III while 'Likely Actionable' and 'Investigational' and prognostic classified as class II. This approach would enable a platform to be supported by multiple regulatory pathways.

Any regulatory proposal also needs to address and mitigate the risk of a wrong test result that does not accurately predict the response to a therapeutic. The NGS Working Group would like to suggest that an appropriate data set that includes a high level of accuracy testing (testing against a reference method, see proposal #5) could adequately address this concern. Of course, certain genomic alterations may require more rigorous review particularly when they are known to be related to safe and effective use of particular treatments. Within this proposal, the working group recognizes there may be exceptions to any broad policy decision on the regulation of genomic oncology panels.

For example, if a drug was targeted against a marker not on the panel then a separate companion diagnostic clearance or approval would need to be sought and classification would be decided based on intended use of the marker. The working group proposes that panel content could be driven by an international public body that would have an unbiased approach to determining panel content and have the appropriate scientific expertise. It will be critical to also include pharmaceutical companies in these discussions as much of the biological significance of markers will be based on work performed as part of drug development efforts. Finally, ensuring that payers are included in the discussion will be important to ensure commercial viability of the approach.

A proposed intended use for such a product could be as follows: The standardized molecular panel is intended for use in identifying specific molecular alterations known to be involved in cancer development and progression as well as known targets for oncology drug development. These

molecular alterations could be used as evidence for clinical study enrollment, if suitable regulatory requirements are met. Molecular alterations that have been cleared or approved to inform specific patient therapeutic treatment can be used for their defined purpose.

One final issue to consider in the development of a regulatory pathway for an FDA cleared or approved cancer panel are the ongoing improvements in technology and changes in content. FDA-cleared panels may quickly become obsolete based on the rapid evolution and discovery in the oncology field. Identifying a mechanism for ensuring rapid inclusion of new markers, performance data for genomic alterations, and updates to assay performance are also needed. One such approach could be allowing new markers designated for clinical trial use only to be added via the 30-Day Notice PMA Supplement process (would require changes to current guidance) or via a new 510(k) should a Class II risk designation be appropriate.

Questions for Discussion for Proposal #1:

a. Which type of organization or professional society could make recommendations on an actionable panel?

b. Could markers without a specific oncology drug listed in its intended use be considered under lower classification (i.e., Class II, 510(k)) than current companion diagnostics? Is it appropriate to consider higher classifications for some markers based on intended use (i.e., efficacy vs. safety)? What risks need to be mitigated in order to consider a 510(k) pathway?

c. Some companion diagnostics have already been approved. Is there a way to bridge the content from the approved CoDx assays onto an NGS panel? Can FDA comment on the nature of the bridging study expectations in the light of it being improbable that complete sets of samples from clinical trials will be available to support bridging studies? Should industry rely on previous approvals for 'me-too' HER2 assays as examples?

d. Should a company 'bridge' the 'me-too' PMA/510(k) content to an NGS panel; could the NGS panel that includes those markers be considered the reference standard moving forward? How could such a panel be adapted for clinical practice?

e. What would the data set be to support an intended use for a non-drug specific companion diagnostic? Would analytical validation be sufficient or would clinical samples be required?

f. What strategies could be considered for handling the mass storage of patient NGS data? How long would/should this data be stored?

g. FDA recently issued the draft guidance: *Expedited Access for Premarket Approval Medical Devices intended for Unmet Medical Need for Life Threatening or irreversibly Debilitating Disease or Conditions – Draft Guidance for Industry and Food and Drug Administration Staff*[4]. Could this guidance be applied to an Actionable Cancer Panel? Could the following be applied to an application for an actionable cancer panel (from Section (3) of the guidance document):

 i. Use of banked samples from previously performed prospective studies.

 ii. Use of literature to support clinical validity?

 iii. Use of contrived samples in some cases?

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[4] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm393879.htm

2. **Proposal:** Approaches for performing validation studies should be based on the types of alterations measured by the assay, rather than every alteration individually.

Office of IVDs and Radiological Products (OIR) has been innovative in the approach to analytical studies for multiplex microbiology panels in the hope of decreasing the analytical study requirements while ensuring the safety of multiplex molecular diagnostic panels. The FDA guidance document: Draft Guidance for Industry and Food and Drug Administration Staff - Highly Multiplexed Microbiological/Medical Countermeasure In Vitro Nucleic Acid Based Diagnostic Devices[5] may be an appropriate reference to consider when determining the analytical requirements for a multiplex cancer panel. In addition, it is important to consider the approach taken in the recent Illumina MiSeqDx clearance of comparing to well-characterized composite reference information derived from the combination of multiple sequencing methodologies, publicly available data, and hereditary information.

The concept of classifying specific types of genomic alterations and performing analytical studies based on those classifications may also be important to consider, given that reference specimens bearing all possible genomic alterations do not exist. For example, it may be appropriate for a multiplex assay that has numerous point mutations to perform studies for point mutations generally, if the various factors that may affect assay performance (such as sequence context) are addressed in the experimental design.

Finally, FDA has historically required some level of validation from the tissue type at which the assay is directed (i.e., lung cancer biopsy if the intended use of the product is for identification of patients for a lung cancer drug) or from blood. While this strategy provides a high degree of assurance of assay performance within each tissue, it may be more important for protein and RNA based assays, rather than for DNA, which is generally less variable. To provide greater flexibility, most NGS validation activities should begin with DNA as the starting specimen rather than the original tissue source, complemented by studies with defined reagents/kits demonstrating reliable DNA extraction across tissue types. Several ongoing or planned studies, including The Cancer Genome Atlas and NCI's MATCH Trial, will add support for reliable DNA retrieval and NGS assay performance from many varieties of tissues.

Questions for Discussion for Proposal #2:

a. What analytical requirements should be considered for an actionable cancer panel?

b. What flexibilities in the level of validation can be adapted for NGS?

c. If an interested group were willing to do a study that examined thoroughly the issue of whether the tissue type effects DNA extraction with defined sets of reagents/kits (i.e., are there known interfering substances in some tumor types that require validation from tissue; across various types of tumors - i.e., breast biopsy, lung biopsy, etc.) could FDA utilize this general study to potentially decrease the validation requirements? Or would this sort of study be required for each specific platform?

d. The handling of massive datasets and developing tools to check and assure sequence quality, conduct sequence alignment and assembly, and biologically interpret and draw inferences from the data remains increasingly important to the implementation of NGS. In establishing performance validation, what requirements are critical to establish data management standards?

e. What requirements should be established specimen pre-analytics (i.e., including tumor cell enrichment)?

f. As other technologies (i.e., whole exome sequencing) are adapted for clinical use, can the definition of a cancer panel be adapted to incorporate a broader genetic assessment?

[5] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm327293.htm

3. **Proposal:** Determine the contents of a cancer panel by classifying potential markers based on current utility in clinical care and clinical trials and peer-reviewed publications as well as recognized clinical guidelines. Similar to the CF example above, draw upon various sources to determine the recommended marker set for an actionable cancer panel.

Rather than attempting to create a specific panel for this paper since there are a variety of groups already working on this issue, the NGS Working Group preparing this paper would like to suggest a process to determining the contents of an actionable cancer panel.

The first step is to choose a way to classify various genomic alterations per cancer type. For example, one approach could be to create four buckets: Known Actionable, Likely Actionable, Investigational (i.e., reasonable hypotheses for significance) and Unknown Significance (may be biologically relevant but no known clinical hypothesis at the time of categorization). Note: markers of unknown significance may not be included on an actionable cancer product but are important to consider during the classification process. It is also an important discussion as to whether markers of unknown significance should be included on a panel but not reported until significance has been determined.

As noted above in the Qiagen EGFR test, approaches that allow for reporting for many markers, even those without demonstrated clinical utility will be important to provide comprehensive information to physicians. Markers would be placed into the appropriate buckets based on the amount of literature available. Only markers which fall into the Known, Likely Actionable and Investigational buckets could be reported on the panel. As markers moved from the Unknown category into either of the other two categories, they would be eligible to move onto the actionable cancer panel products.

The NGS Working Group further proposes that this list should be modified at least annually. The process for reclassifying a marker should be similar to the original classification process. Performance standards for each marker or group of markers will need to be established. Performance for FDA cleared or approved panels will be publically available and the NGS Working Group encourages each CLIA lab to also publish performance data for its LDTs so that comparisons of all cancer panels, regardless of where it was developed, can be generated. The NGS Working Group would also like to encourage reimbursement organizations to require publication of this data as well.

Questions for Discussion for Proposal #3:

a. Is it desirable to have a single list of markers to measure for the entire industry?

b. How should performance standards for each marker or group of markers be established?

c. Should markers be stratified/tiered based on level of evidence and tumor type to guide their inclusion in a panel (i.e. availability of clinical data; biologically relevant versus exploratory)?

d. Can a panel accommodate complex use of the data? For example, if one wanted to use a gene panel to "score" tumor mutation burden?

4. **Proposal:** Promote the standardization of cancer panels through the development and use of a common set of samples to ensure reproducibility on each platform.

For all assays whether they are FDA cleared/approved or LDTs not subject to clearance or approval, we propose that a common set of samples be prepared which test various types of mutations (i.e., insertion/deletions, mutations, rearrangements, etc.) to ensure data is available to show performance of each platform. More specifically, the minimum performance isn't mandated, rather, the NGS Working Group encourages transparency of the data so that decisions on the appropriate product to use can be made based on data.

One of the challenging issues with developing panels of standardized materials is determining how they will eventually be replenished. Since a standardization panel, based on well-characterized tissue specimens, would be challenging to prepare and replenish, an approach of creating mock specimens should be considered.

Questions for Discussion for Proposal #4:

a. Which international organization or group would prepare such samples? How should the work that is currently being performed by NIST and NCI be considered for this proposal?

b. How could access to such a sample set be guaranteed?

c. Could this type of standardization of assays result in more standard reimbursement? Does having a particular set of data make it easier for the reimbursement bodies to make decisions? Could the results of the proficiency testing be made publicly available?

5. **Proposal:** Establish a framework for determining the appropriate reference method rather than relying on any single reference method for all studies.

Central to all of the discussions in this paper is finding agreement on an appropriate reference method as well as determining what approach should be taken when a reference method is not available.

Note: A distinction is made between reference methods and methods that are utilized to resolve discordant results between the original assay result and the reference method. Whether a method can be called a reference method or a discordant resolution method depends on how the analysis is performed. If all samples are tested a priori on two methods then both may be considered reference methods. However, if a third method is utilized but only discordant samples are tested than that method is not considered a reference method.

As noted above, each of the current panels have used different orthogonal reference methods in an attempt to ensure 'truth' is reported. OIR has traditionally requested bi-directional Sanger sequencing as the preferred reference method even though it is becoming more often less sensitive than other methods including PCR and NGS. Please see the following table of recent FDA approved CoDx products and the use of reference and discordant resolution methods:

Product Name	Sample Type	Reference Method for Accuracy Study	Discordant Method
Roche BRAF	FFPE Melanoma	Sanger sequencing	% mutation determined by alternate sequencing method
bMX BRAF	FFPE Melanoma	Sanger sequencing	NA
Qiagen EGFR	FFPE NSCLC	Sanger sequencing	NA
Roche EGFR	FFPE NSCLC	Sanger sequencing and Massively Parallel Sequencing (MPS)	NA
Qiagen KRAS	FFPE CRC	Sanger sequencing	NA

The following data summaries from the Roche EGFR PMA demonstrate that where methods in addition to bi-directional Sanger sequencing are used it is obvious that more sensitive reference methods are already necessary and as NGS assays continue to improve will become more important.

Roche cobas EGFR Mutation Test; PMA Summary P120019 (pages 7 and 8)
A total of 406 samples with valid cobas test and Sanger sequencing results were included in the agreement analysis. The agreement analysis between the cobas® EGFR Mutation Test results and Sanger sequencing results for the detection of the EGFR mutation demonstrated a PPA of 96.6%, a NPA of 88.3%, and an OPA of 90.6%. The PPA, NPA, and OPA in the detection of exon 19 deletion mutations were all greater than 92%. The PPA, NPA, and OPA in the detection of L858R mutations compared were all greater than 95%.

A total of 408 samples with valid cobas test and MPS results were included in the agreement analysis. The agreement analysis between the cobas® EGFR Mutation Test results and MPS sequencing results for the detection of the EGFR mutation demonstrated a PPA of 94.0%, a NPA of 97.7%, and an OPA of 96.3%. The PPA, NPA, and OPA in the detection of exon 19 deletion mutations were all greater than 95%. The PPA, NPA, and OPA in the detection of L858R mutations were all greater than 90%.

Rather than trying to agree upon a single reference method or simply creating bi-directional Sanger sequencing as a standard, the NGS Working Group proposes examining the following questions for agreeing on an appropriate method:

Proposed Reference Method Decision Questions

* *What is the analyte being measured?*
  Assays measuring the same analyte are more attractive reference methods. For example, while FISH as a measure of amplification may be appropriate as a reference method for NGS copy number determination, IHC may be less desirable as only the protein consequences of the alteration are measured. For quantitative analyses, quantitative reference methods such as PCR, FISH, or another comparative array method may be attractive.

- *What is the sensitivity of the NGS assay system relative to proposed reference method?*
  To verify the positive predictive value (PPV) of NGS findings, only reference methods with comparable sensitivity may be used. For example, if the NGS assay has sensitivity down to the 5% minor allele level, bi-directional Sanger sequencing, with sensitivity at a much higher level cannot be used as a confirmatory assay. On the other hand, if in this example the analytical validation experiment seeks to establish NGS detection of variants previously observed using bi-directional sequencing (i.e. establishing the sensitivity of the NGS assay to at least the bi-directional level), the use of bi-directional Sanger sequencing as a reference method may be appropriate.

- *What is the regulatory status of the proposed reference method?*
  If a proposed reference method meeting the criteria above has previously been reviewed and cleared by FDA, this method should be used. However, if no FDA approved reference method is available with the appropriate performance characteristics, other reference methods may be considered, provided that sufficient information is provided to FDA.

- *What if no absolute reference method available?*
  There are examples to draw from in this situation:

  1) Use of composite data:

     a. The Illumina MiSeq clearance to well-characterized composite reference information derived from the combination of multiple sequencing methodologies, publicly available data, and hereditary information.

     b. Comparison and presentation of data from multiple sources as in the Affymetrix CytoScan clearance. Comparison against a next generation sequencing method is presented in addition to comparison of historical IHC/FISH data and clinical performance data.

  2) Reliance on the demonstration of reproducibility and precision (rather than accuracy) as has been done with IHC CoDx assays

Questions for Discussion for Proposal #5:

a. What principles should be applied in the selection of an appropriate reference method?

b. Under what circumstances can composite data serve as the reference method? What properties of composite data are required to serve as a reference method?

c. Under what circumstances can the reference method requirement be waived?

d. On the issue of sensitivity, what mechanism is appropriate to demonstrate the Limit of Detection (LOD) of NGS panels?

Current FDA Device Classification[6] Reference guide:

Investigational device exemption (IDE)

An IDE allows the investigational device to be used in a clinical study in order to collect safety and effectiveness data required to support a Premarket Approval (PMA) application or a Premarket Notification 510(k) submission to FDA. Clinical studies with devices of significant risk must be approved by FDA and by an Institutional Review Board (IRB) before the study can begin. Studies with devices of non-significant risk must be approved by the IRB only before the study can begin.

Devices classified as Class I or II, and if it is not exempt, a 510k will be required for marketing. Classification is risk based, that is, the risk the device poses to the patient and/or the user is a major factor in the class it is assigned. Class I includes devices with the lowest risk and Class III includes those with the greatest risk.

- Class I devices are deemed to be low risk and are therefore subject to the least regulatory controls.

- Class II devices are higher risk devices than Class I and require greater regulatory controls to provide reasonable assurance of the device's safety and effectiveness.

- Class III devices are those that support or sustain human life, are of substantial importance in preventing impairment of human health, or which present a potential, unreasonable risk of illness or injury.

Premarket approval (PMA)

Products requiring PMAs are Class III devices that pose a significant risk of illness or injury, or devices found not substantially equivalent to Class I and II predicates (although some may go through a de novo process)

Premarket notification 510(k)

A 510(k) is a premarket submission made to FDA to demonstrate that the device to be marketed is at least as safe and effective, that is, substantially equivalent, to a legally marketed device that is not subject to PMA.

- Same intended use

- Same technology or new technology that does not raise new questions of safety and effectiveness

Data required to support substantial equivalence varies significantly depending on the type of device; it may or may not require clinical trials.
CLIA (applicable to IVDs)

- CLIA refers to the Clinical Laboratory Improvement Amendments of 1988, which establishes quality standards for laboratory testing. CMS assumes primary responsibility for regulation under CLIA.

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[6] http://www.fda.gov/medicaldevices/deviceregulationandguidance

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